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EP-A- 0 110 294
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CHEMICAL ABSTRACTS, vol. 81, no. 7, 19th August 1974, page 77, abstract no. 33770b, Columbus, Ohio, US; A.I. DOBROSOK et al.: "Action of parathyroid hormone and calcium-45 on the bone tissue of rats", & BYULL. EKSP. BIOL. MED. 1974, 77(4), 112-115

Endo et al., Nature, 286: 262-264 (1980)

Clinical Chemistry in Diagnosis and Treatment (1981)

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Biochemie, Karlson pages 231, 335 (1980)

Mutschler, Arzneimittelwirkungen, page 220 (1975)

Labhart Klinik der Inneren Sekretion, p. 827-845 (1978)

Clinical Science, Hetti et al., 62, p. 389-396 (1982)

Description

The present invention relates to a pharmaceutical combination for increasing bone mass in a human afflicted with osteoporosis.

Osteoporosis is a generic description for a group of diverse diseases which are characterized by a reduction in the mass of bone per unit volume with a histologically normal ratio of osteoid to bone. The effects of these diseases are particularly severe when the mass per unit volume decreases to a level below that required for adequate mechanical support. Osteoporosis is a particularly important cause of morbidity in the elderly. The most frequent symptoms are back pain and deformity of the spine resulting from a collapse of the vertebrae, especially in the lumbar and thoracic spine regions.

The problem presented worldwide by osteoporosis is truly staggering. It is estimated that in the United States alone, there are millions who symptomatically exhibit this disease. The disease appears to be an invariable accompaniment of aging, particularly among women, and the incidence is increasing with the age expectancy of the population. Osteoporosis often occurs when there is malnutrition, as a complication due to immobilization and paralysis, as a result of liver, kidney, or intestinal disease, and also after or during various forms of endocrine or rheumatic diseases. As improved public health measures increase life expectancy in third world nations and under-developed countries, the incidence of osteoporosis is beginning to be recognized as a significant problem in these countries as well. For these reasons, a treatment that can arrest the course of this widely present, destructive disease represents a significant medical advance.

Various methods have been discussed in the prior art for increasing bone mass in humans with osteoporosis. These treatments include administration of sodium fluoride, androgens, biologically active forms of parathyroid hormone alone, calcitonin, and calcitonin in combination with high dietary phosphate. Except for treatment with sodium fluoride, the effects of these treatments are modest. Sodium fluoride treatment increases trabecular bone in some patients but has uncertain effects on total bone mass and bone strength, a high risk of osteomalacia, as well as other unpleasant side effects.

In addition to these methods of treating osteoporosis by increasing bone mass, other methods are known which are designed to preserve existing bone mass. These methods involve the use of estrogens or calcium alone, or 1-hydroxy vitamin D_3 or 1,25-dihydroxy vitamin D_3 alone.

Typical publications disclosing and discussing these prior art methods include the following. Brugger et al., U.S. Patent 3,956,260, discloses the preparation and use of a synthetic polypeptide for the treatment of osteoporosis. This polypeptide is unrelated to that of the present invention. Christie et al., U.S. Patent 4,241,051, teaches topical application of the hormone calcitonin for the treatment of diseased bone in the ear.

Reeve et al., British Medical Journal, 280:1340 (1980), describes the results of a multicenter trial evaluating the effect of a fragment of human parathyroid hormone (hPTH (1-34)) on osteoporosis in humans. The authors report that this regimen resulted in a considerable increase in axial trabecular bone, but calcium retention, and hence, total bone mass, improved in only about half of these patients. In other patients calcium retention worsened and therefore, total bone mass decreased. The authors speculate that hPTH(1-34) might be most effective if administered in combination with estrogen, calcitonin, a diphosphonate, or some other agent that would limit resorption while allowing bone formation to continue.

Reeve et al., in Monoclonal Antibodies And Developments In Immunoassay, p. 239, published by Elsevier/North-Holland Biomedical Press (1981), report their progress towards answering some of the questions raised in the above study. The authors state that their studies of intestinal calcium absorption point to a possible defect in osteoporosis and further speculate that it may be necessary to circumvent this defect with, for example, 1,25-$(OH)_2$ vitamin D_3 in modest dosage, given during intervals when no hPTH injection is administered.

Hefti et al., Clinical Science, 62:389 (1982), describes studies using a normal calcium diet supplemented with either parathyroid hormone or 1,25-$(OH)_2$ vitamin D_3 using normal and osteoporotic adult rats. The authors report that, although these studies showed an increase of whole-body calcium and skeletal mass, there was no restoration of individual trabeculae lost during the development of osteoporosis. Endo et al., Nature, 286:262 (1980), discuss the use of metabolites of vitamin D in conjunction with parathyroid hormone (PTH) to stimulate bone formation in vitro. However, these treatments with PTH and 1,25-$(OH)_2$ vitamin D_3 were no more effective than PTH alone in stimulating re-calcification of bone.

Rader et al., Calcified Tissue International, 29(1):21 (1979), describes the treatment of thyroparathyroidectomized rats with dietary calcium and intraperitoneal injection of a parathyroid extract. Although this treatment stimulated 1,25-$(OH)_2$ vitamin D_3 production and effected a marked increase in bone mineralization, it was also found to produce bone resorption as evidenced by the appearance of cavities in the cortical bone. There was no effect on rates of bone formation, or bone matrix apposition.

Wong et al., Surgical Forum, 30:100 (1979), teach the administration to thyroparathyroidectomized dogs of daily intramuscular parathyroid extract or oral 1,25-$(OH)_2$ vitamin D_3 simultaneously with thyroid replacement therapy. The effect of these treatments on absorption of dietary calcium is discussed in the context of parathyroidism although not in the context of osteoporosis.

Peacock et al., Vitamin D Proceedings Workshop., E. Norman, Ed., p. 411 (1977), disclose the inhibition by calcitonin and steroid sex hormones of the resorptive effect of vitamin D metabolites and parathyroid hormone on mouse calvaria bone in tissue culture. Pechet et al., American Journal of Medicine, 43(5):696 (1967), teach that minimum levels of parathyroid hormone are necessary in order for vitamin D to exert its effects on bone resorption rather than bone formation. In Mahgoub et al., Biochemical and Biophysical Research Communications, 62:901 (1975), the authors describe experiments and state that active vitamin D metabolites (25-OH vitamin D_3 and 1,25-$(OH)_2$ vitamin D_3) potentiate the ability of parathyroid hormone to elevate the cyclic AMP levels of cultured rat fetal bone cells.

None of these methods, however, have provided a clinically useful technique for treating osteoporosis and related disorders and often cause undesirable side effects. As a consequence, there is still a need for a generally effective treatment having minimal side effects.

Accordingly, it is the object of this invention to provide a pharmaceutical composition which can be used for increasing bone mass in a human afflicted with osteoporosis.

This object of the invention has been accomplished by providing a pharmaceutical composition for increasing bone mass in a human which comprises a parathyroid hormone or physiologically active fragment thereof, or equivalent thereof, in combination with (a) a single hydroxylated vitamin D compound, or a structural or functional analogue thereof, or (b) a dietary calcium supplement.

Figure 1 shows the changes in trabecular bone density accompanying therapy according to the invention in five adult males with idiopathic osteoporosis.

The inventors have devised a pharmaceutical combination for increasing bone mass which represents a significant improvement over the prior art compositions intended to accomplish this effect. The present invention comprises the administration, to a human afflicted with osteoporosis, of a combination of a parathyroid hormone or physiologically active fragment thereof, with either a single hydroxylated vitamin D derivative, or a structural or functional analogue thereof, or a dietary calcium supplement.

The parathyroid hormone appears to stimulate bone formation. The hydroxylated vitamin D component seems to act both to increase the intestinal absorption of calcium and to positively affect bone metabolism either directly or through its effects on calcium absorption from the intestines. Alternatively, a high calcium dietary supplement appears to increase calcium absorption from the intestines, thereby shifting the equilibrium of bone formation and bone resorption in favor of bone formation. The synergistic effects of these treatments are demonstrated hereinafter in clinical studies.

The present invention is intended to be used in all diseases classified as osteoporosis, particularly postmenopausal osteoporosis, senile osteoporosis, idiopathic osteoporosis, immobilization osteoporosis, postpartum osteoporosis, juvenile osteoporosis, and osteoporosis secondary to gonadal insufficiency, malnutrition, hyperprolactinemia, prolactinoma, disorders of the gastrointestinal tract, liver, or kidneys, and osteoporosis that is a sequella of prior osteomalacia, chronic acidosis, thyrotoxicosis, hyperparathyroidism, glucocorticoid excess or chronic disorders involving the bone marrow, and heritable forms of osteoporosis such as osteogenesis imperfecta and its variants, and other heritable disorders of connective tissue.

The first component involved in the method of the invention is a "parathyroid hormone," or fragment thereof hereafter abbreviated "PTHF." PTHF may consist of the first twenty-six, twenty-eight, thirty-four or any other physiologically active number of amino acids (numbered from the amino terminal) of a parathyroid hormone obtainable from a human or other vertebrate.

The term "obtainable" is intended to indicate that the PTHF is not necessarily derived from animal-produced parathyroid hormone but may be synthetically made, based on a natural model.

The term "fragment" is not intended to eliminate compounds larger or smaller than those specifically shown, but is intended to include all components obtainable from naturally occurring parathyroid hormones, up to and including or beyond the natural compounds themselves.

PTHF also encompasses chemically modified parathyroid hormone fragments which retain the activity associated with parathyroid hormone. The necessary activity is the stimulation of bone formation. Modifications that may be considered include:

(1) PTHF with carboxyl amino acid extensions beyond position 34 up to or beyond position 84 of the human PTH molecule, or amino-terminal extensions, or amino acid substitutions that produce other desirable features, such as an alpha-carboxyl amide at the carboxyl terminus. A desirable modification should delay metabolism and/or enhance activity in vivo;

(2) PTHF extended to include amino acids 1-38, which would enhance receptor binding and hence the

activity per mole;

(3) PTHF with D-amino acid substitutions so as to delay metabolism and thereby enhance potency in vivo;

(4) PTHF chemically modified so as to enhance its absorption through the skin, mucous membranes, or gastro-intestinal tract to avoid thereby the necessity of parenteral injection; and

(5) physiologically acceptable salts and esters of PTHF.

A PTHF obtainable from a mammal (PTHF 1-34) is generally preferred over other types of parathyroid hormone fragments, such as derivatives. Use of a PTHF consisting of the first thirty-four amino acid residues of human parathyroid hormone (hereafter abbreviated "hPTHF 1-34") is especially preferred for use in humans. Other preferred PTHF's are those which display some or all of the following desirable features: increased potency with regard to the necessary activity, increased ease of administration, increased selectivity to decrease potential side effects, and decreased antigenicity in humans to avoid an adverse immune response. PTHF molecules having the following formulas are particularly preferred:

```
     1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-
 10                  15
Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-
 20                  25
Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-
 30
Asp-Val-His-Asn-Phe-COOH
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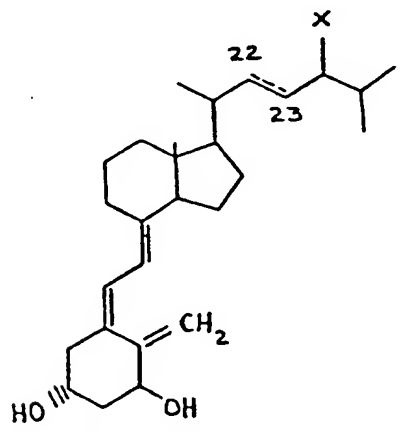
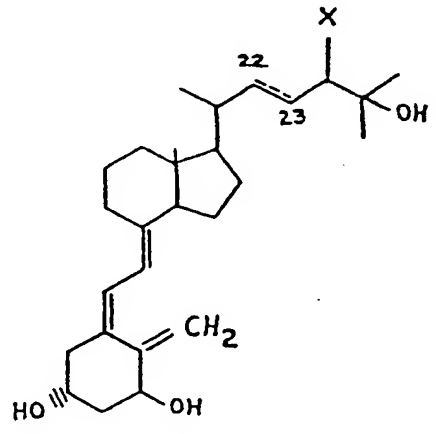
Ranges of administration of hPTHF 1-34, for example, are 100-700 units/day, more preferably 200-600 units/day, and most preferably 400-500 units/day, wherein "units" are defined in terms of the International Reference Preparation of hPTHF 1-34 and comparative bioassays in one of the established PTH bioassays. Potency ratios of different PTH analogues differ in different assays. The "units" are expressed in the chick hypercalcemic assay.

For other PTHF molecules, the ranges of administration are those high enough to stimulate bone remodeling in humans, yet not so high as to produce net bone resorption nor enough bone mineral mobilization to produce hypercalcemia or hypercalciuria. For compounds other than hPTH 1-34, dosage can be quantitated on a weight basis, or in terms of an appropriately established reference standard.

Hydroxylated vitamin D compounds of the invention include 1-alpha-hydroxy vitamin D_3 and 1-alpha,25-dihydroxy vitamin D_3 and their vitamin D_2 analogues: 1-alpha-hydroxy vitamin D_2 and 1-alpha,25-dihydroxy vitamin D_2. These molecules have the following formulas:



1-alpha-hydroxy vitamin D



1-alpha,25-dihydroxy vitamin D

wherein the bond between C-22 and C-23 may be single or double and wherein X may be H or -CH_3.

Chemical variations which retain the characteristics of these hydroxylated vitamin D molecules are contemplated as equivalents. Preferred are those like the glucuronide and sulfate derivatives of the above, dihydrotachysterol, 5,6-trans forms of vitamin D, and all other forms of vitamin D capable of stimulating the intestinal absorption of calcium without the necessity for prior hydroxylation at carbon 1 by the patient. Most preferred are compounds which also stimulate absorption of calcium for brief periods of time (days rather than weeks) after their administration is discontinued. These latter compounds are useful because they permit rapid reduction in biological effect in the event of overdose. Very large doses of vitamin D and 25-OH vitamin D stimulate the intestinal absorption of calcium without prior hydroxylation at carbon 1, but the resultant biological effects often last for weeks or months after administration of the drug has been halted, thus exposing the patient to a risk of toxicity.

The dose ranges for the administration of the vitamin D component are those large enough to produce the characteristic effects of vitamin D, particularly an enhanced intestinal absorption of calcium in osteoporotic patients, but not so large as to produce hypercalciuria or hypercalcemia. Possible dose ranges of various vitamin D analogues are illustrated in Table 1.

Table 1

Vitamin D Analogue	RANGE[a] Broad	Preferred	Most Preferred
1-alpha,25-$(OH)_2$ Vit D_3 or 1-alpha,25-$(OH)_2$ Vit D_2	0.05-2.0	0.1-1.0	0.25-0.50
1-alpha-OH Vit D_3 or 1-alpha-OH Vit D_2	0.05-3.0	0.1-1.5	0.25-0.75
25-OH Vit D_3 or 25-OH Vit D_2	10-150	20-100	20-50
Vit D_3 or Vit D_2	1250 mcg. every 3 days to 3750 mcg/day.	1250 mcg on alternate days to 2500 mcg/day.	1250 mcg 3 x weekly
dihydrotachysterol	0.2-1.2 mg/day	0.2-0.6 mg/day	0.2-0.4 mg/day

[a] Units in mcg/day unless otherwise noted.

By "dietary calcium supplement" as used in this invention is meant supplementing the normal diet with calcium at a level greater than that level which is recommended as the daily dietary allowance. In normal adult humans, the recommended daily allowance is 20-25 millimoles calcium/day and slightly higher in postmenopausal women, yet the customary intake of calcium among adults in the U.S. is only 12-18 millimoles/day. Since in many osteoporotic humans, the intestines are inefficient in absorbing calcium, this sub-optimal calcium diet merely serves to aggravate their osteoporosis. Accordingly, a dietary calcium

supplement for an adult would involve the administration of sufficient calcium to increase the total oral intake of diet plus supplement to 38-50 millimoles/day. When a dietary calcium supplement is used, the calcium is administered in a non-toxic form. The dosage rates mentioned herein refer to the amounts of calcium present, and the dosage rate of the actual compound used can be easily calculated therefrom using the formula weight of the compound being administered. Milk or any non-toxic salt of calcium may be utilized provided that the counter ion is not toxic to the human in which it is being administered. Typical suitable non-toxic counter ions include carbonate, citrate, phosphate, gluconate, lactate, chloride, and glycerol phosphate. The upper limit of the dietary calcium supplement is determined by the toxic effects of calcium, which varies slightly from patient to patient, as is well understood by those skilled in the art. Typically, in humans, the maximum allowance per day is 2000 mg calcium per day.

Use of the method of the invention is aided by pharmaceutical combinations comprising the ingredients intended for use in the method of the invention. Such pharmaceutical combinations were not suggested by the prior art since the method of the invention, which involves using specific combinations of ingredients, was unknown to the prior art.

By "pharmaceutical combination" as used herein is meant to include intimate mixtures of the two components of the invention, as in classical compositions, and also non-mixed associations, such as those found in kits or pharmaceutical packs.

A typical pharmaceutical mixed composition of the invention would contain PTHF in combination with a hydroxylated vitamin D compound or calcium in a form suitable for use as a dietary calcium supplement. The composition may additionally contain a pharmaceutically acceptable carrier and, if intended for oral administration, may comprise the PTHF in a liposome to protect this component.

The components can be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermal absorption, and orally. Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance drug absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending the liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the components of the invention, the medicament being used for increasing bone mass.

The materials for use in the administration of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes and the like, each of said container means comprising one of the separate elements to be used in the method as well as such means as syringes and needles, sprays or dermal patches for administration of said elements.

For example, one of the container means may contain parathyroid hormone fragment (1-34) in lyophilized form or in solution. A second container may comprise a single hydroxylated vitamin D compound or a dietary calcium supplement in tablet form or in solution. The carrier means may also contain a third container means comprising a buffer solution for rehydrating lyophilized components present in the kit.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE 1

The effects of the methods and compositions of the invention were investigated in human patients. Three adult males afflicted with osteoporosis were given 500 units of a human parathyroid hormone fragment (hPTHF 1-34) and 0.25 µg of 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3) daily for a period of six to twelve months while consuming a normal calcium diet (15-20 mmoles/day). A fourth patient was given the same amount of parathyroid hormone fragment while receiving a high calcium intake (total intake more than 50 mmoles Ca/day). The effects of these treatments on calcium and phosphorus balances are shown in Table 2. The effect of these treatments on bone density is shown in Table 3.

The balance measurements involve complete collection and chemical analysis of all excreta for 18 days, during which the patient ate exactly the same foods each day. The minerals excreted were compared

to those ingested to determine the total body retention, or "balance," for each patient. Cortical bone density was determined in the forearm by measuring the attenuation of radioactive ^{125}I photons by the shaft of the radius. Trabecular bone density was determined in the spine by quantitative computed x-ray tomography measurements of the lumbar vertebral bodies. These techniques are widely used for such purposes by those of skill in the art.

Table 2

	Patient 1			Patient 2			Patient 3			Patient 4		
	hPTH + 1,25-$(OH)_2$ D			hPTH + 1,25-$(OH)_2$ D			hPTH + 1,25-$(OH)_2$ D			hPTH + Ca > 2 grams		
	Pre	Rx	Delta	Pre	Rx	Delta	Pre	Rx	Delta	Pre	Rx	Delta
Calcium												
Serum (mg%)	9.19	9.44	+0.25	9.24	8.75	-.49	9.7	9.3	-0.4	9.32	9.46	+0.14
Urine (mg/d)	255	400	+145	191	243	+52	237	297	+60	288	372	+84
Balance (mg/d)	-114	-61	+53	-320	+114	+434	-110	-223	-113	-199	+131	+330
Net Absorption (mg/d)	136	339	+203	-129	345	+474	103	123	+23	90	499	+409
Phosphorus												
Serum (mg%)	4.34	3.99	-.35	3.52	3.56	+.04	4.5	4.0	-0.5	3.55	3.59	+0.04
Urine (mg/d)	1028	1073	+45	805	548	-257	459	768	-309	1339	1400	+61
Balance (mg/d)	-254	-142	+112	-262	+213	+475	173	-90	-263	+33	+188	+155
Net Absorption (mg/d)	774	931	+157	542	745	+203	632	679	+47	1372	1587	+215

An improvement in calcium balance can be noted in Table 2. Failure to observe consistent improvement in calcium balance was one negative factor previously experienced with parathyroid hormone when it was administered without effort to augment intestinal absorption of calcium beyond provision of a diet containing the recommended dietary allowance for calcium.

Serial maintenance of cortical bone density in the forearm of three of these same patients are shown in Table 3. The control measurements were made over several months for each individual, and the values on treatment also reflect measurements made at intervals at several months each.

Table 3

Bone Densitometry 1/3 Distal (g/cm^2)

	Patient 1	Patient 2	Patient 3	Patient 4
Control	0.75	0.74	0.80*	.625
	0.75	0.79	0.74	.63
	--	0.78	--	.613
On Rx	hPTH + $(1,25\text{-}(OH)_2$ D)	hPTH + $(1,25\text{-}(OH)_2$ D)	hPTH + $(1,25\text{-}(OH)_2$ D)	hPTH + Ca
	0.67	0.79	0.71	.61
	0.71	0.82	0.71	.65
	0.69	0.83	0.69	.60
	0.72	--	0.73	.67
	0.74	--	0.70	--
Off Rx	0.75 (off Rx x 4 mos.)	0.83 (off Rx x 1 yr.)	--	--

* 2-1/2 years prior to initiation of treatment

Computerized tomography was used to measure bone density in the spine of patient 4. These measurements were performed on three lumbar vertebral (L1 through L3). A single 1 cm thick section was obtained through the mid-vertebral body using the scout view for localization. After the first series of scans, the patient was allowed to move freely before the duplicate set of measurements was taken. The results of the scans are shown in Table 4.

Table 4

	Reading 1	Reading 2
Bone Density After 11 Months Of Treatment		
L1	91.1	90.7
L2	88.0	90.2
L3	83.2	90.7
Bone Density After 17 Months Of Treatment		
L1	111	107
L2	99	107
L3	105	97
Bone Density at End of Treatment		
L1	104	109
L2	110	104
L3	106	107

The bone densities shown in Table 4 which were taken after eleven months of treatment are approximately 2 standard deviations below the mean for a man of this patient's age. By comparison, bone density measurements made at the end of treatment according to the method of the invention show an increase in bone density over the nine-month treatment interval of approximately 20%.

EXAMPLE 2

Five adult males with idiopathic osteoporosis were treated with hPTHF(1-34) and 1,25-$(OH)_2$ D_3 - (patients 5-8) or with hPTHF(1-34) and calcium (patient 4) using dosages as described in Example 1. The results of this study are presented graphically in Figure 1. The trabecular bone density measurements in individual lumbar vertebral bodies are expressed as K_2HPO_4 equivalents. All patients showed a significant increase in vertebral trabecular bone density during treatment.

Patient 4 showed a steady increase in bone densities until the twentieth month of therapy, at which time treatment was discontinued. When bone densities were measured fourteen months after the cessation of therapy, this patient's bone density had again declined. This further indicates the effectiveness of the combined therapy according to the invention in reversing the effects of osteoporosis on trabecular bone density of the vertebrae.

In patients 7 and 8, it was impossible to measure several of the vertebrae because of vertebral fractures preceding treatment. These figures denote a progressive and consistent improvement in the trabecular bone density of these patients.

Cortical bone density was measured in the forearm of these same patients prior to treatment and every three months during treatment. The density measurements showed no consistent change.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

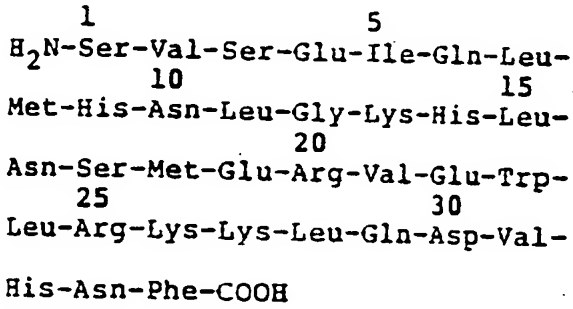
1. A pharmaceutical combination for use in increasing bone mass in a patient in need thereof comprising a parathyroid hormone or physiologically active fragment thereof, in combination with:
   (a) a single hydroxylated vitamin D compound, or
   (b) a non-toxic calcium salt.

2. The combination according to claim 1, wherein said combination is an intimate mixture.

3. The combination of claim 1 or 2, wherein said parathyroid hormone fragment is a peptide consisting of the first 34 amino acid residues of a parathyroid hormone obtainable from a human or animal.

4. The combination of claim 3, wherein said hormone fragment is obtainable from a human.

5. The combination of claim 3 or 4, wherein said fragment has the amino acid sequence:



6. The combination of any one of claims 1 to 5, wherein said combination comprises from 100 to 700, preferably from 200 to 600, most preferably from 400 to 500 units of said parathyroid hormone fragment.

7. The combination of any one of claims 1 to 6, wherein said combination comprises 1-hydroxy Vitamin D or 1,25 dihydroxy Vitamin D.

8. The combination of any one of claims 1 to 7, wherein said combination contains from 0.05 to 150 mcg, preferably from 0.05 to 3.0 mcg, most preferably from 0.05 to 2.0 mcg of said Vitamin D compound.

9. The combination of any one of claims 1 to 6, wherein said combination comprises calcium coupled to a non-toxic counter ion.

10. The combination of claim 9, wherein said non-toxic counter ion is carbonate, citrate, phosphate, gluconate, lactate, chloride or glycerol phosphate.

11. The combination of any one of claims 1 to 6 or 9 to 10, wherein said combination contains from 12.5 to 50 mmoles of said calcium salt.

12. The combination of claim 11, wherein said calcium salt is calcium carbonate.

13. The combination of any one of claims 9 to 12 comprising a non-toxic calcium compound which is to be administered at a rate of no greater than 2,000 mg/day in order to achieve a high calcium intake of 38-50 mmoles/day in adults.

14. A kit useful for administering a bone mass increasing composition comprising a carrier being compartmentalized to receive in close confinement therein one or more containers wherein:
(a) a first container or series of containers contain parathyroid hormone obtainable from a human, or other animal or a physiologically active fragment thereof;
(b) a second container contains
(A) a single hydroxylated vitamin D compound, or
(B) a non-toxic calcium salt, and
(c) a third container contains a buffer for reconstituting or diluting components of said kit.

15. The kit of claim 14, wherein said kit contains means for administering the content of said first container

and the content of said second container.

16. The kit of claim 14 or 15, wherein said first container contains a peptide fragment of said parathyroid hormone, as defined in claims 3 to 5.

17. The kit of any one of claims 14 to 16 wherein said hydroxylated Vitamin D compound is 1-hydroxy Vitamin D or 1,25-dihydroxy Vitamin D.

18. The kit of claim 14, wherein said non-toxic calcium salt is a dietary calcium supplement.

Claims for the following Contracting State : AT

1. A process for the preparation of a pharmaceutical combination for use in increasing bone mass in a patient in need thereof which comprises mixing a parathyroid hormone or physiologically active fragment thereof with:
   (a) a single hydroxylated vitamin D compound, or
   (b) a non-toxic calcium salt.

2. The process according to claim 1, wherein mixing is is carried out in such a way that an intimate mixture is obtained.

3. The process of claim 1 or 2, wherein said parathyroid hormone fragment is a peptide consisting of the first 34 amino acid residues of a parathyroid hormone obtainable from a human or animal.

4. The process of claim 3, wherein said hormone fragment is obtainable from a human.

5. The combination of claim 3 or 4, wherein said fragment has the amino acid sequence:

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     1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-
         10                  15
Met-His-Asn-Leu-Gly-Lys-His-Leu-
                    20
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-
    25                  30
Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-

His-Asn-Phe-COOH
```

6. The process of any one of claims 1 to 5, wherein said combination comprises from 100 to 700, preferably from 200 to 600, most preferably from 400 to 500 units of said parathyroid hormone fragment.

7. The process of any one of claims 1 to 6, wherein said combination comprises 1-hydroxy Vitamin D or 1,25 dihydroxy Vitamin D.

8. The process of any one of claims 1 to 7, wherein said combination contains from 0.05 to 150 mcg, preferably from 0.05 to 3.0 mcg, most preferably from 0.05 to 2.0 mcg of said Vitamin D compound.

9. The process of any one of claims 1 to 6, wherein said combination comprises calcium coupled to a non-toxic counter ion.

10. The process of claim 9, wherein said non-toxic counter ion is carbonate, citrate, phosphate, gluconate, lactate, chloride or glycerol phosphate.

11. The process of any one of claims 1 to 6 or 9 to 10, wherein said combination contains from 12.5 to 50 mmoles of said calcium salt.

12. The process of claim 11, wherein said calcium salt is calcium carbonate.

13. The process of any one of claims 9 to 12 wherein the combination comprises a non-toxic calcium compound which is to be administered at a rate of no greater than 2,000 mg/day in order to achieve a high calcium intake of 38-50 mmoles/day in adults.

14. A kit useful for administering a bone mass increasing composition comprising a carrier being compartmentalized to receive in close confinement therein one or more containers wherein:
(a) a first container or series of containers contain parathyroid hormone obtainable from a human, or other animal or a physiologically active fragment thereof;
(b) a second container contains
(A) a single hydroxylated vitamin D compound, or
(B) a non-toxic calcium salt, and
(c) a third container contains a buffer for reconstituting or diluting components of said kit.

15. The kit of claim 14, wherein said kit contains means for administering the content of said first container and the content of said second container.

16. The kit of claim 14 or 15, wherein said first container contains a peptide fragment of said parathyroid hormone, as defined in claims 3 to 5.

17. The kit of any one of claims 14 to 16 wherein said hydroxylated Vitamin D compound is 1-hydroxy Vitamin D or 1,25-dihydroxy Vitamin D.

18. The kit of claim 14, wherein said non-toxic calcium salt is a dietary calcium supplement.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Combinaison pharmaceutique utilisable pour augmenter la masse osseuse chez un patient nécessitant un tel traitement, comprenant une hormone parathyroïdienne ou un fragment physiologiquement actif de celle-ci, en combinaison avec : (a) un seul composé de vitamine D hydroxylé, ou (b) un sel de calcium non toxique.

2. Combinaison suivant la revendication 1, caractérisée en ce que la combinaison précitée est un mélange intime.

3. Combinaison suivant l'une ou l'autre des revendications 1 et 2, caractérisée en ce que le fragment d'hormone parathyroïdienne est un peptide formé des 34 premiers restes d'acides aminés d'une hormone parathyroïdienne obtenable chez un être humain ou un animal.

4. Combinaison suivant la revendication 3, caractérisée en ce que le fragment d'hormone est obtenable chez un être humain.

5. Combinaison suivant l'une ou l'autre des revendications 3 et 4, caractérisée en ce que le fragment précité a la séquence d'acides aminés :

```
     1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-
         10                  15
Met-His-Asn-Leu-Gly-Lys-His-Leu-
                    20
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-
     25                  30
Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-

His-Asn-Phe-COOH
```

6. Combinaison suivant l'une quelconque des revendications 1 à 5, caractérisée en ce que ladite combinaison comprend de 100 à 700, avantageusement de 200 à 600, plus avantageusement de 400 à 500 unités du fragment d'hormone parathyroïdienne précité.

7. Combinaison suivant l'une quelconque des revendications 1 à 6, caractérisée en ce que ladite combinaison comprend de la 1-hydroxy vitamine D ou de la 1,25-dihydroxy vitamine D.

8. Combinaison suivant l'une quelconque des revendications 1 à 7, caractérisée en ce que ladite combinaison contient de 0,05 à 150 mcg, avantageusement de 0,05 à 3,0 mcg, plus avantageusement de 0,05 à 2,0 mcg du composé de vitamine D.

9. Combinaison suivant l'une quelconque des revendications 1 à 6, caractérisée en ce que ladite combinaison comprend du calcium couplé à un contre-ion non toxique.

10. Combinaison suivant la revendication 9, caractérisée en ce que le contre-ion non toxique est du carbonate, du citrate, du phosphate, du gluconate, du lactate, du chlorure ou du glycérol phosphate.

11. Combinaison suivant l'une quelconque des revendications 1 à 6 ou 9 à 10, caractérisée en ce que ladite combinaison contient de 12,5 à 50 mmoles du sel de calcium précité.

12. Combinaison suivant la revendication 11, caractérisée en ce que le sel de calcium est du carbonate de calcium.

13. Combinaison suivant l'une quelconque des revendications 9 à 12, comprenant un composé de calcium non toxique qui est à administrer à un taux ne dépassant pas 2.000 mg/jour afin de réaliser une absorption de calcium élevée de 38-50 mmoles/jour chez des adultes.

14. Trousse utilisable pour administrer une composition augmentant la masse osseuse, comprenant un support compartimentalisé pour y loger de manière étroitement confinée un ou plusieurs récipients, dans laquelle :
    (a) un premier récipient ou une première série de récipients contiennent de l'hormone parathyroïdienne obtenable chez un être humain ou tout autre animal ou un fragment physiologiquement actif de celle-ci;
    (b) un second récipient contient
        (A) un seul composé de vitamine D hydroxylé, ou
        (B) un sel de calcium non toxique, et
    (c) un troisième récipient contient un tampon pour reconstituer ou diluer les composants de ladite trousse.

15. Trousse suivant la revendication 14, caractérisée en ce que ladite trousse contient un moyen pour administrer le contenu du premier récipient et le contenu du second récipient.

16. Trousse suivant l'une ou l'autre des revendications 14 et 15, caractérisée en ce que le premier récipient contient un fragment peptidique de l'hormone parathyroïdienne, tel que défini aux revendications 3 à 5.

17. Trousse suivant l'une quelconque des revendications 14 à 16, caractérisée en ce que le composé de vitamine D hydroxylé est de la 1-hydroxy vitamine D ou de la 1,25-dihydroxy vitamine D.

18. Trousse suivant la revendication 14, caractérisée en ce que le sel de calcium non toxique est un supplément de calcium alimentaire.

Revendications pour l'Etat contractant suivant : AT

1. Procédé pour la préparation d'une combinaison pharmaceutique utilisable pour augmenter la masse osseuse chez un patient nécessitant un tel traitement, qui comprend le mélange d'une hormone parathyroïdienne ou d'un fragment physiologiquement actif de celle-ci avec :
   (a) un seul composé de vitamine D hydroxylé, ou
   (b) un sel de calcium non toxique.

2. Procédé suivant la revendication 1, caractérisé en ce que le mélange est réalisé d'une manière telle qu'un mélange intime est obtenu.

3. Procédé suivant l'une ou l'autre des revendications 1 et 2, caractérisé en ce que le fragment d'hormone parathyroïdienne est un peptide formé des 34 premiers restes d'acides aminés d'une hormone parathyroïdienne obtenable chez un être humain ou un animal.

4. Procédé suivant la revendication 3, caractérisé en ce que le fragment d'hormone est obtenable chez un être humain.

5. Procédé suivant l'une ou l'autre des revendications 3 et 4, caractérisé en ce que le fragment précité a la séquence d'acides aminés :

```
     1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-
          10                  15
Met-His-Asn-Leu-Gly-Lys-His-Leu-
                    20
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-
     25                  30
Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-

His-Asn-Phe-COOH
```

6. Procédé suivant l'une quelconque des revendications 1 à 5, caractérisé en ce que ladite combinaison comprend de 100 à 700, avantageusement de 200 à 600, plus avantageusement de 400 à 500 unités du fragment d'hormone parathyroïdienne précité.

7. Procédé suivant l'une quelconque des revendications 1 à 6, caractérisé en ce que ladite combinaison comprend de la 1-hydroxy vitamine D ou de la 1,25-dihydroxy vitamine D.

8. Procédé suivant l'une quelconque des revendications 1 à 7, caractérisé en ce que ladite combinaison contient de 0,05 à 150 mcg, avantageusement de 0,05 à 3,0 mcg, plus avantageusement de 0,05 à 2,0 mcg du composé de vitamine D.

9. Procédé suivant l'une quelconque des revendications 1 à 6, caractérisé en ce que ladite combinaison comprend du calcium couplé à un contre-ion non toxique.

10. Procédé suivant la revendication 9, caractérisé en ce que le contre-ion non toxique est du carbonate, du citrate, du phosphate, du gluconate, du lactate, du chlorure ou du glycérol phosphate.

11. Procédé suivant l'une quelconque des revendications 1 à 6 ou 9 à 10, caractérisé en ce que ladite

combinaison contient de 12,5 à 50 mmoles du sel de calcium précité.

12. Procédé suivant la revendication 11, caractérisé en ce que le sel de calcium est du carbonate de calcium.

13. Procédé suivant l'une quelconque des revendications 9 à 12, caractérisé en ce que la combinaison comprend un composé de calcium non toxique qui est à administrer à un taux ne dépassant 2.000 mg/jour afin de réaliser une absorption de calcium élevée de 38-50 mmoles/jour chez des adultes.

14. Trousse utilisable pour administrer une composition augmentant la masse osseuse, comprenant un support compartimentalisé pour y loger de manière étroitement confinée un ou plusieurs récipients, dans laquelle :
(a) un premier récipient ou une première série de récipients contiennent de l'hormone parathyroïdienne obtenable chez un être humain ou tout autre animal ou un fragment physiologiquement actif de celle-ci;
(b) un second récipient contient
(A) un seul composé de vitamine D hydroxylé, ou
(B) un sel de calcium non toxique, et
(c) un troisième récipient contient un tampon pour reconstituer ou diluer les composants de ladite trousse.

15. Trousse suivant la revendication 14, caractérisée en ce que ladite trousse contient un moyen pour administrer le contenu du premier récipient et le contenu du second récipient.

16. Trousse suivant l'une ou l'autre des revendications 14 et 15, caractérisée en ce que le premier récipient contient un fragment peptidique de l'hormone parathyroïdienne, tel que défini aux revendications 3 à 5.

17. Trousse suivant l'une quelconque des revendications 14 à 16, caractérisée en ce que le composé de vitamine D hydroxylé est de la 1-hydroxy vitamine D ou de la 1,25-dihydroxy vitamine D.

18. Trousse suivant la revendication 14, caractérisée en ce que le sel de calcium non toxique est un supplément de calcium alimentaire.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Pharmazeutische Kombination zur Verwendung in der Vermehrung von Knochenmasse in einem bedürftigen Patienten, umfassend ein Parathyroidhormon oder ein physiologisch aktives Fragment davon, in Kombination mit:
(a) einer einzelnen hydroxylierten Vitamin D-Verbindung, oder
(b) einem nicht-toxischen Calciumsalz.

2. Kombination nach Anspruch 1, wobei die Kombination ein inniges Gemisch ist.

3. Kombination nach Anspruch 1 oder 2, wobei das Parathyroidhormon-Fragment ein Peptid ist, das aus den ersten 34 Aminosäureresten eines von einem Menschen oder Tier erhältlichen Parathyroidhormons besteht.

4. Kombination nach Anspruch 3, wobei das Hormonfragment von einem Menschen erhältlich ist.

5. Kombination nach Anspruch 3 oder 4, wobei das Fragment die folgende Aminosäuresequenz hat:

```
    1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-
        10                  15
Met-His-Asn-Leu-Gly-Lys-His-Leu-
                20
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-
    25                  30
Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-

His-Asn-Phe-COOH
```

6. Kombination nach einem der Ansprüche 1 bis 5, wobei die Kombination 100 bis 700, vorzugsweise 200 bis 600, besonders bevorzugt 400 bis 500 Einheiten des Parathyroidhormon-Fragments umfaßt.

7. Kombination nach einem der Ansprüche 1 bis 6, wobei die Kombination 1-Hydroxy-Vitamin D oder 1,25-Dihydroxy-Vitamin D umfaßt.

8. Kombination nach einem der Ansprüche 1 bis 7, wobei die Kombination 0,05 bis 150 µg, vorzugsweise 0,05 bis 3,0 µg, besonders bevorzugt 0,05 bis 2,0 µg der Vitamin D-Verbindung enthält.

9. Kombination nach einem der Ansprüche 1 bis 6, wobei die Kombination ein an ein nicht-toxisches Gegenion gekuppeltes Calcium umfaßt.

10. Kombination nach Anspruch 9, wobei das nicht-toxische Gegenion Carbonat, Citrat, Phosphat, Gluconat, Lactat, Chlorid oder Glycerinphosphat ist.

11. Kombination nach einem der Ansprüche 1 bis 6 oder 9 bis 10, wobei die Kombination 12,5 bis 50 mMole des Calciumsalzes enthält.

12. Kombination nach Anspruch 11, wobei das Calciumsalz Calciumcarbonat ist.

13. Kombination nach einem der Ansprüche 9 bis 12, umfassend eine nicht-toxische Calciumverbindung, die mit einer Rate verabreicht werden soll, die nicht größer als 2000 mg/Tag ist, um eine hohe Calciumaufnahme von 38 bis 50 mMol/Tag in Erwachsenen zu erreichen.

14. Kit, der für die Verabreichung eines die Knochenmasse vermehrenden Mittels wertvoll ist, umfassend einen Träger, der in Fächer eingeteilt ist, so daß eng nebeneinander darin ein oder mehrere Behälter enthalten sind, worin:
    (a) ein erster Behälter oder eine Reihe von Behältern Parathyroidhormon enthalten, welches von einem Menschen oder einem anderen Tier erhältlich ist, oder ein physiologisch aktives Fragment davon;
    (b) ein zweiter Behälter
        (A) eine einzelne hydroxylierte Vitamin D-Verbindung, oder
        (B) ein nicht-toxisches Calciumsalz enthält, und
    (c) ein dritter Behälter einen Puffer für die Rekonstitution oder Verdünnung von Bestandteilen des Kits enthält.

15. Kit nach Anspruch 14, wobei der Kit Mittel für die Verabreichung des Inhalts des ersten Behälters und des Inhalts des zweiten Behälters enthält.

16. Kit nach Anspruch 14 oder 15, wobei der erste Behälter ein Peptidfragment des Parathyroidhormons gemäß der Definition in den Ansprüchen 3 bis 5 enthält.

17. Kit nach einem der Ansprüche 14 bis 16, wobei die hydroxylierte Vitamin D-Verbindung 1-Hydroxy-Vitamin D oder 1,25-Dihydroxy-Vitamin D ist.

18. Kit nach Anspruch 14, wobei das nicht-toxische Calciumsalz ein diätetischer Calciumzusatz ist.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung einer pharmazeutischen Kombination zur Verwendung in der Vermehrung von Knochenmasse in einem bedürftigen Patienten, umfassend das Zusammengeben eines Parathyroidhormons oder eines physiologisch aktiven Fragments davon mit:
   (a) einer einzelnen hydroxylierten Vitamin D-Verbindung, oder
   (b) einem nicht-toxischen Calciumsalz.

2. Verfahren nach Anspruch 1, wobei das Zusammengeben so durchgeführt wird, daß ein inniges Gemisch erhalten wird.

3. Verfahren nach Anspruch 1 oder 2, wobei das Parathyroidhormon-Fragment ein Peptid ist, das aus den ersten 34 Aminosäureresten eines von einem Menschen oder Tier erhältlichen Parathyroidhormons besteht.

4. Verfahren nach Anspruch 3, wobei das Hormonfragment von einem Menschen erhältlich ist.

5. Verfahren nach Anspruch 3 oder 4, wobei das Fragment die folgende Aminosäuresequenz hat:

```
    1                   5
H2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-
        10                  15
Met-His-Asn-Leu-Gly-Lys-His-Leu-
                20
Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-
    25                  30
Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-

His-Asn-Phe-COOH
```

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei die Kombination 100 bis 700, vorzugsweise 200 bis 600, besonders bevorzugt 400 bis 500 Einheiten des Parathyroidhormon-Fragments umfaßt.

7. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Kombination 1-Hydroxy-Vitamin D oder 1,25-Dihydroxy-Vitamin D umfaßt.

8. Verfahren nach einem der Ansprüche 1 bis 7, wobei die Kombination 0,05 bis 150 µg, vorzugsweise 0,05 bis 3,0 µg, besonders bevorzugt 0,05 bis 2,0 µg der Vitamin D-Verbindung enthält.

9. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Kombination ein an ein nicht-toxisches Gegenion gekuppeltes Calcium umfaßt.

10. Verfahren nach Anspruch 9, wobei das nicht-toxische Gegenion Carbonat, Citrat, Phosphat, Gluconat, Lactat, Chlorid oder Glycerinphosphat ist.

11. Verfahren nach einem der Ansprüche 1 bis 6 oder 9 bis 10, wobei die Kombination 12,5 bis 50 mMole des Calciumsalzes enthält.

12. Verfahren nach Anspruch 11, wobei das Calciumsalz Calciumcarbonat ist.

13. Verfahren nach einem der Ansprüche 9 bis 12, wobei die Kombination eine nicht-toxische Calciumverbindung enthält, die mit einer Rate verabreicht werden soll, die nicht größer als 2000 mg/Tag ist, um eine hohe Calciumaufnahme von 38 bis 50 mMol/Tag in Erwachsenen zu erreichen.

14. Kit, der für die Verabreichung eines die Knochenmasse vermehrenden Mittels wertvoll ist, umfassend einen Träger, der in Fächer eingeteilt ist, so daß eng nebeneinander darin ein oder mehrere Behälter enthalten sind, worin:
    (a) ein erster Behälter oder eine Reihe von Behältern Parathyroidhormon enthalten, welches von einem Menschen oder einem anderen Tier erhältlich ist, oder ein physiologisch aktives Fragment davon;
    (b) ein zweiter Behälter
        (A) eine einzelne hydroxylierte Vitamin D-Verbindung, oder
        (B) ein nicht-toxisches Calciumsalz enthält, und
    (c) ein dritter Behälter einen Puffer für die Rekonstitution oder Verdünnung von Bestandteilen des Kits enthält.

15. Kit nach Anspruch 14, wobei der Kit Mittel für die Verabreichung des Inhalts des ersten Behälters und des Inhalts des zweiten Behälters enthält.

16. Kit nach Anspruch 14 oder 15, wobei der erste Behälter ein Peptidfragment des Parathyroidhormons gemäß der Definition in den Ansprüchen 3 bis 5 enthält.

17. Kit nach einem der Ansprüche 14 bis 16, wobei die hydroxylierte Vitamin D-Verbindung 1-Hydroxy-Vitamin D oder 1,25-Dihydroxy-Vitamin D ist.

18. Kit nach Anspruch 14, wobei das nicht-toxische Calciumsalz ein diätetischer Calciumzusatz ist.

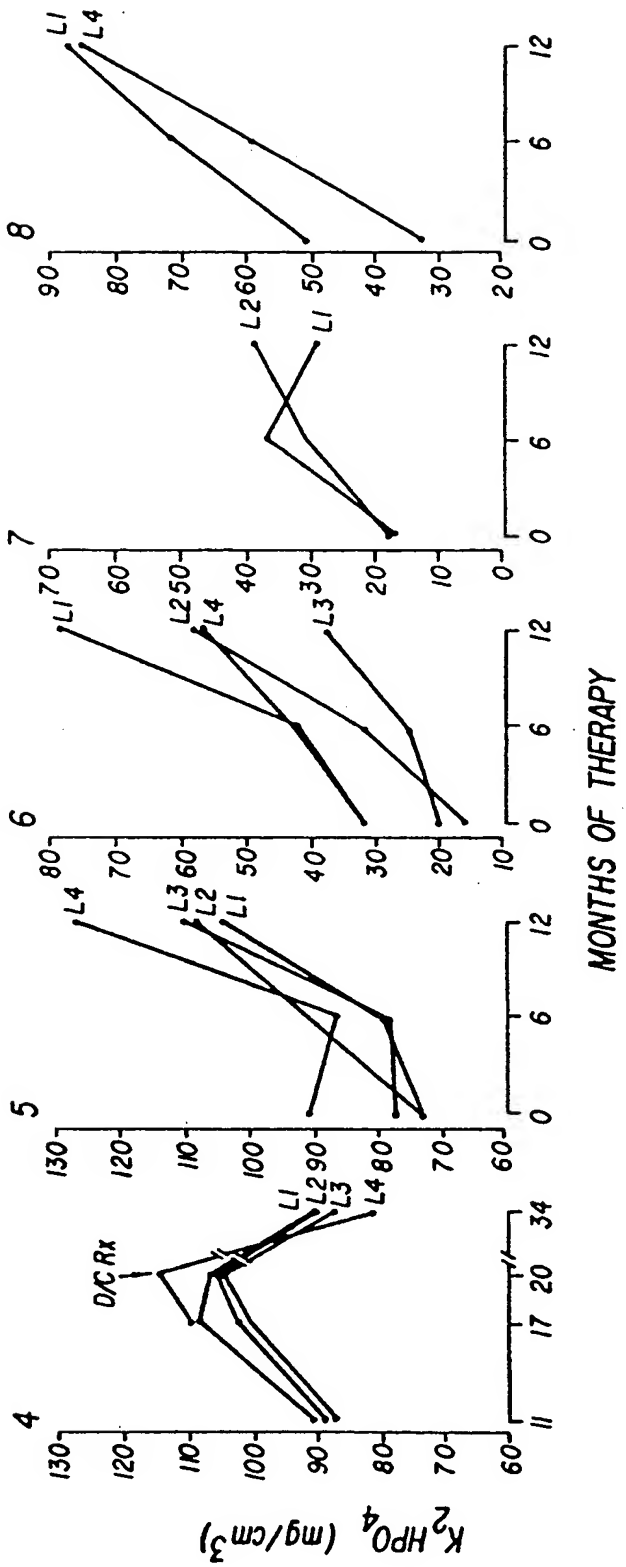

FIG. 1